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14. ABSTRACT

Equipment Purchase - A Q-sense E4 Auto Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) with was bought to support DoD-relevant research on structural proteins, molecular interactions and surface science in general. The equipment included the following components:

- Complete Q-sense E4 Auto QCM-D system, fully equipped with standard accessories (including MHz standard gold crystals) and analysis system (including computer and monitor)

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a. REPORT	b. ABSTRACT	c. THIS PAGE			David Kaplan
UU	UU	UU	UU		19b. TELEPHONE NUMBER 617-627-3251

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Report Title

Final Report: Quartz Crystal Microbalance with Dissipation Monitoring

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- optional modules: Ellipsometry Module, Electrochemistry Module (including reference electrode & accessories), Window Module, Open Module, Humidity Module, Standard Flow Module (extra)
- UV/Ozone cleaner
- Hydroxyapatite, 10 nm, Sensors
- Biotin Functionalized on Gold Sensors
- His-tag Capturing Sensor

QCM-D techniques provide answers about mass and structural changes at the nanoscale level. The instrumentation was used to characterize the interaction between small molecules or polymers and a designed interface, to assess the hydration states of surface coatings, conformational changes during assembly and further material interactions. The equipment was used mostly for the investigation of interfaces and processes involving silk or silk-based peptides and inorganic components, to quantify loading and binding of molecules involved in the development sensors, to analyze functionalized materials for interactions, to study bio-nanocomposite systems and to investigate block copolymer self–assembly to form nanostructures.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

N/A

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper

TOTAL:

Number of Manuscripts:

Books

Received   Book

TOTAL:

Received   Book Chapter

TOTAL:

Patents Submitted

N/A

Patents Awarded

N/A

Awards

N/A

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Faculty Supported

NAME PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Names of Under Graduate students supported

NAME PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:...... 0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:...... 0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:...... 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

See Attachment

Technology Transfer

N/A

Final Report, W911NF-13-1-0262 – Quartz Crystal Microbalance with Dissipation Monitoring
Tufts University, PI: David L. Kaplan

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<u>Research Activity with Equipment</u> - A brief summary of the research that has been performed with the equipment is reported in the following sections.

<u>Binding of recombinant collagen-like molecules to collagen</u> - We have previously constructed a recombinant collagen like molecules with Fn binding sequence insertion from human collagen type II (VCL-Fn). The Fn affinity has been shown by an ELISA-like solid-state binding assay. We were trying to confirm the binding affinity and potentially get more quantitative binding data using QCMD as an alternative technique. Initially the Au sensor was used to our experiments. Collagen solutions were pumped through the sensor surface for the first layer coating, after thorough washing steps, Fn solution was pumped through the sensor as the second layer. We were able to observe the first frequency drop which indicated the collagen molecules were coated onto the surface efficiently. However, we failed to observe any frequency drop when Fn solutions were pumped through. Several tries have been made with varied conditions but without positive results. A His-tag binding sensor was used as an alternative choice because the recombinant VCL-Fn carries a his-tag for purification. By using this chip, we managed to detect the second frequency drop (**Figure 1**). The pump speed was set at the default 300ul/min, which based on the curve is too fast for our experiement, because the solution ran out before the curve reached saturation. We ended up stop the pump to let the Fn solution incubate on the sensor for a half hour. This experiment will be performed again with much slower pump speed (10ul/min).

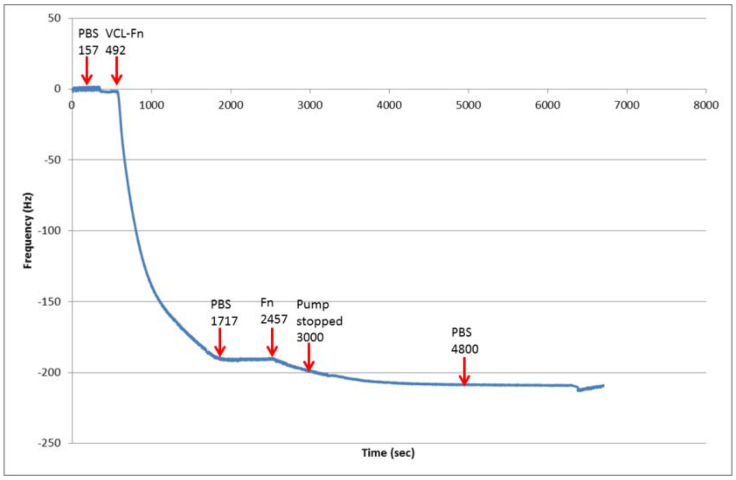

Figure 1. Fn affinity of VCL-Fn with His-tag sensor

<u>Study of the pH sensitive properties of silk-inspired triblock protein</u> - A silk-inspired triblock protein, termed $C_cE_{20}C_c$ (**Figure 2**) was engineered with a long glutamic acid (Glu)-abundant E_{20} block (red, containing 40 mol % of Glu) in the middle and joined at the two ends by short, pH-switchable, supramolecular crosslinking C_c blocks (blue). The E_{20} block consists of 20 repeats of a 10-residue repetitive motif, EEPETPSPET, found in *Nephila clavipes* aggregate silk glue. The 28-amino-acids-long C_c block, IAALEAENEALKAEIAELKAEIAAEKAE, was selected after screening a few *de novo* designed peptide candidates. At any pH greater than the pKa of the Glu side-chains, the negative charge predominates in either of the constituent blocks, and the chimeric protein as a whole adopts a disordered and extended conformation. When pH decreases to below the pKa, Glu side-chains are protonated. Hence, the neutral E_{20} block switches to a random coil conformation, while the neutral C_c blocks aggregate into α-helical coiled-coil crosslinks. QCM-D tests of proteins, both $C_cE_{20}C_c$ and the middle block E_{20}, were performed using a standard flow cell and the results were summarized in **Figure 3**.

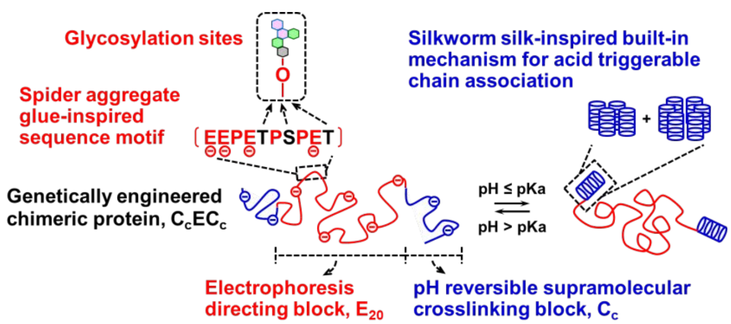

Figure 2. Scheme of chimeric design with three functional domains: two C_c and one E_{20}

The proteins were immobilized onto the gold sensor in citric buffer (pH = 4), rinsed and washed subsequently in cycles of citric buffer (pH = 4) and PBS buffer (pH = 7.4). Multiple pH cycles demonstrated that the adsorbed protein layers exhibited respectively, higher dissipation (softer) in neutral pH and lower dissipation (tougher) at acidic pH, with excellent reversibility up to five cycles. At pH = 7.4, a higher dissipation was observed in the triblock protein $C_cE_{20}C_c$ with respect to the E_{20} counterparts, probably due to both the increased molecular weight in $C_cE_{20}C_c$ and the enhanced interchain interaction contributed by the hydrophobic C_c domains. Overall, these results were consistent with circular dichroism and UV-visible measurements.

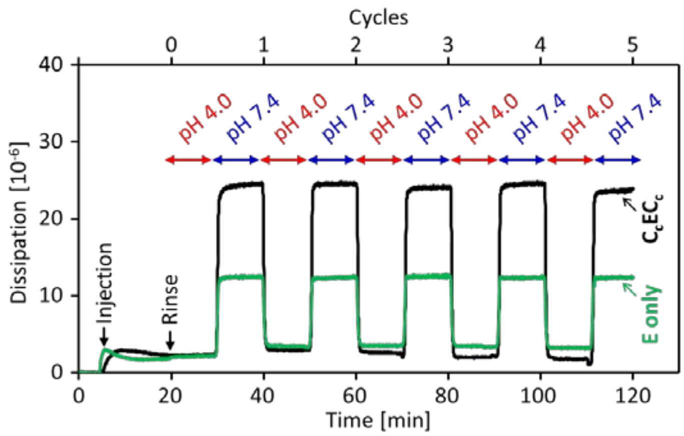

Figure 3. Representative curve of dissipation change during pH cycles.

Characterization of water absorption rate on magnesium films and silk films. The humidity module was used to measure water absorption rate on magnesium and silk films. Gold sensors were coated with a 30 nm film of Mg by e-beam evaporation, or a 50 nm film of silk by spin coating, and different saturated salt solutions were used to achieve different humidities. Different saturated salt solutions were flowed on the upper chamber of the module. Water vapor can diffuse through the membrane in the module and equilibrate in the lower chamber (where the sensor sits). Using different salts, different humidity can be achieved in the lower chamber ranging from 11 % (saturated LiCl solution) to 100 % (DI water). Because of membrane failure and sample failure no dataset was aquired yet. New experiments are ongoing to set up the proper experimental condition and sample preparation.

Caracterization of Silk-elastin like protein (SELP) and chemotherapeutics binding. Silk-elastin like protiens (SELPs) with different affinity tags were expressed and purified. Silk blocks (GAGAGS) mimicking the natural silkworm heavy chain tend to assemble into insoluble tightly packed secondary structures, β-sheets, whereas elastin blocks (GXGVP) are hydrophilic below the inverse transition temperature and stumili responsive. SE8Y, which has 1 silk-block and 8 elastin blocks in an SELP monomer, has a low affinity to the Au sensor. hSE8Y with a His-tag in the SELP monomer, and cSE8Y with a cistine modified his-tag had better gold affinity (**Figure 4**).

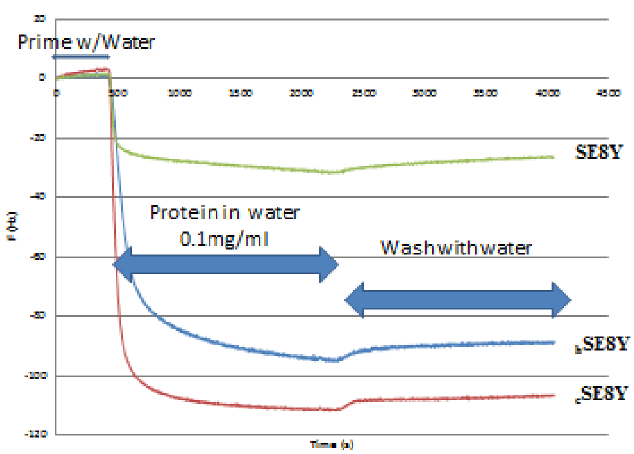

Figure 4. Gold affinity of silk-elastin like protien with different affinity tag.

Silk-elastin like protein (SELP) and small molecular chemicals were studied in terms of binding by QCM. The Au sensor was pre-coated with silk-elastin like protein (SELP), and doxrubicin binding with the SELP was tested by QCM (**Figure 5**). Doxrubicin, which has a positive surface charge, bound to the SELP, which has a slightly negtive surface charge, due to the electrostatic force.

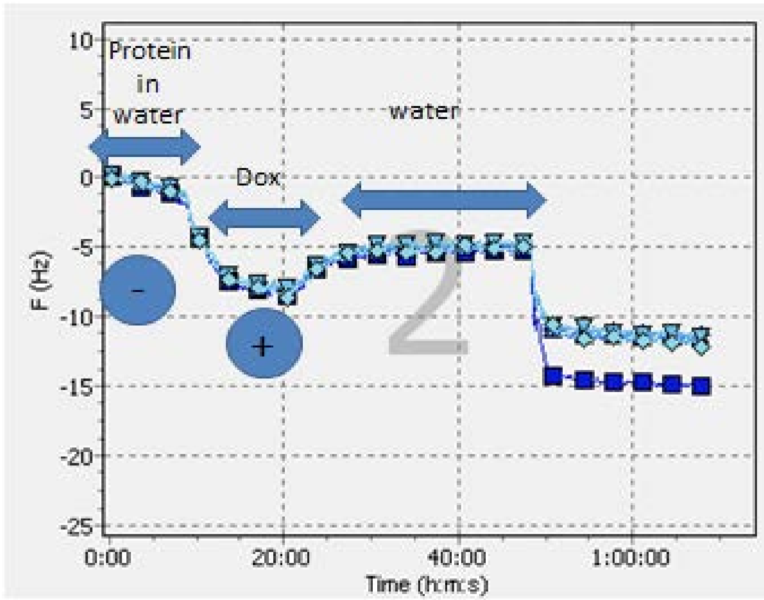

Figure 5. Small molecule binding by silk-elastin like proteins (SELPs).